Title: **ARTIFICIAL SUBSTRATE (PFU) DEPLOYMENT AND RETRIEVAL**

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1.0 OBJECTIVE

Polyurethane foam units (PFUs) are designed for the capture of aquatic microbial assemblages. The purpose of this method is to collect microbial assemblages from the field using artificial substrates (PFUs) for site assessment or for use in laboratory experiments. This method is derived from Cairns et al. 1979, 1986.

2.0 HEALTH AND SAFETY

Protective footwear should always be worn in the field. Additional personnel protective equipment should be worn if sampling from an extremely contaminated site.

3.0 PERSONNEL/TRAINING/RESPONSIBILITIES

Personnel should not perform this method until training by experienced individuals is complete.

4.0 REQUIRED AND RECOMMENDED MATERIALS

glass sample jars or ziploc bags
polyurethane foam cut into 4x5x6 cm cubes
3-hole bricks or other anchoring device
cotton string
nylon rope
gallon-size ziploc bags

cooler
knife or scissors

5.0 PROCEDURE

5.1 Artificial Substrate Deployment

- PFUs should be tied to a brick, with the length of the string adjusted such that the PFUs are positioned approximately 12 inches from the water surface at low tide. Transects of PFUs can be created using 2 bricks connected with nylon rope and the PFUs tied along the rope.

- PFUs should be placed at the same depth at all sites. Position the PFUs where they will remain submerged and undisturbed during their exposure. Mark their location so you will be able to find them when you collect.

- Water quality measurements should be taken at time of deployment.

- See QA/QC section for comments on length of PFU exposure.

5.2 Artificial Substrate Retrieval

- Label the collection containers.

- Keeping the PFUs underwater, cut the string from the brick and gently slide each PFU into a plastic ziploc bag, sterile 8 oz. glass sample jar, or other appropriate container.

- The container should be filled with water from the site, brought to the surface and sealed. The containers should be placed in a cooler (cold but not frozen) and transported to the laboratory. PFUs can also be packed on ice and shipped overnight to an off-site location.

- After returning to the laboratory, open the container, pour off the excess water, and squeeze the contents of each PFU into a sterile sample cup. Each substrate yields approximately 60 mL volume.

- Samples should be processed immediately upon receipt. Samples are homogenized by gently stirring the contents and aliquots are removed for various microbial analyses (see appropriate SOPs).

6.0 QUALITY CONTROL/QUALITY ASSURANCE

A minimum of three replicates per site or treatment is recommended. Confirming the appropriate exposure duration is essential to assure that a representative microbial sample is collected. Before beginning a monitoring program or experimental manipulation, slides should be made from PFU samples exposed over a range of time (e.g. 4, 7, 10, 14 days). After placing a fixed volume of sample on a glass slide, algae and protozoa present in each sample should be identified to genus under a total magnification of 500. Slides should made and viewed until no new taxa are found. A graph of the number of taxa versus exposure time will produce an asymptotic curve. The point where the curve levels off indicates the exposure period required for a representative sample.

If a survey of different sites is the goal, then an effort should be made to avoid pseudoreplication. For example, PFUs could be placed at different locations within each site, but it will be debatable as to whether they constitute true replicates. It will depend on the degree of interaction they encounter in the water body. If a transplant experiment is performed, then it is essential to mock transplant some PFUs back to the initial site. They should be evaluated to assure that the transplant itself did not alter the microbial community.

Also to minimize variability and erroneous results, analyses should be performed as soon as possible after retrieval of the PFUs. Measurements may be biased if the organisms are given time to die or reproduce. Frequently, there will be no significant change in numbers or kinds of species when samples are analyzed within 48-60 hours of collection. PFUs should be collected carefully so as not to release the contents, and they should be transported to the laboratory as soon as possible.

7.0 REFERENCES

Cairns, J., D.L. Kuhn, & J.L. Plafkin. 1979. Protozoan colonization of artificial substrates. Pp. 39-57 In *Methods and Measurement of Periphyton Communities: A Review*. R.L. Wietzel, ed., American Society for Testing and Materials, Philadelphia.

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